Bacillus anthracis Antimicrobials Derived from Inhibitors of Mammalian Serine-Threonine Kinases

---

Jimmy D. Ballard, Ph.D.
PHF Presidential Professor
The Department of Microbiology and Immunology
The University of Oklahoma Health Sciences Center

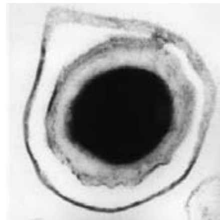
Bacillus anthracis - Host Interactions

WRCE *B. anthracis* Investigators:

- **Theresa Koehler, University of Texas – Houston**
- **Jimmy Ballard, University of Oklahoma HSC**
- **Steven Blanke, University of Illinois**
- **C. Rick Lyons, University of New Mexico HSC**

NIH/ Regional Centers for Excellence-Biodefense and Emerging Infectious Diseases
U54 AI057156-01

Steps in Pathogenesis of Inhalation Anthrax



Entry of spores
into lungs

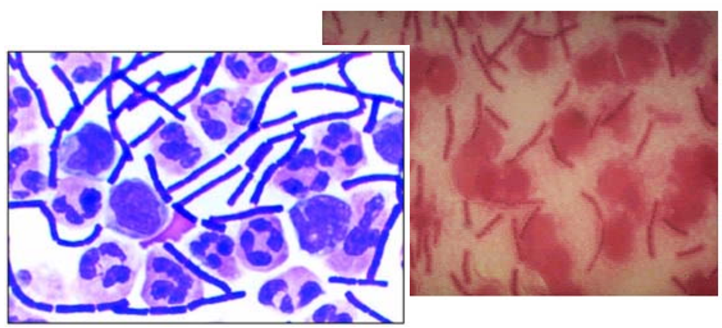
Germination

Transport to mediastinal
lymph nodes by
alveolar macrophages

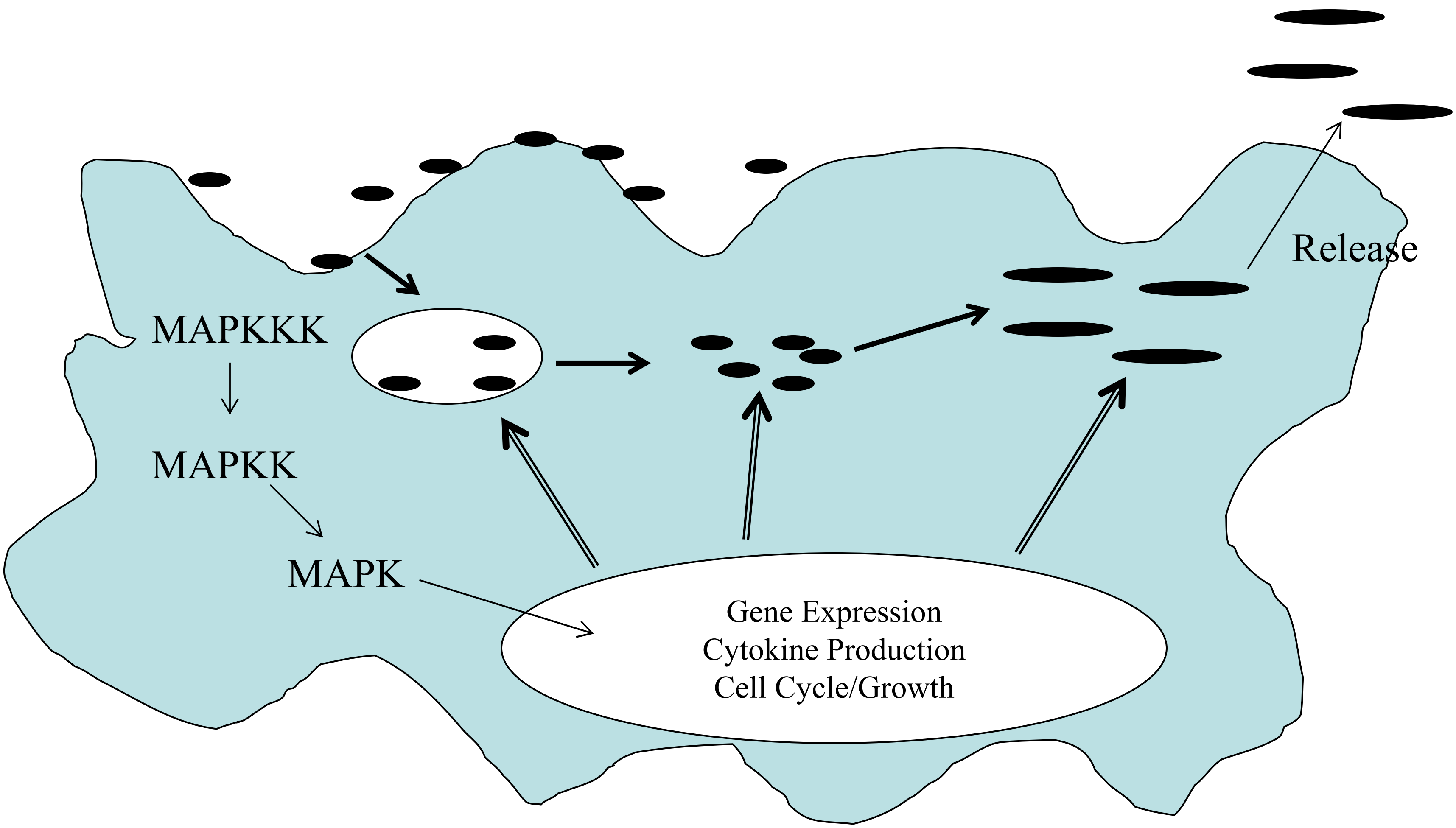
Capsule
& Toxin
Synthesis

Multiplication

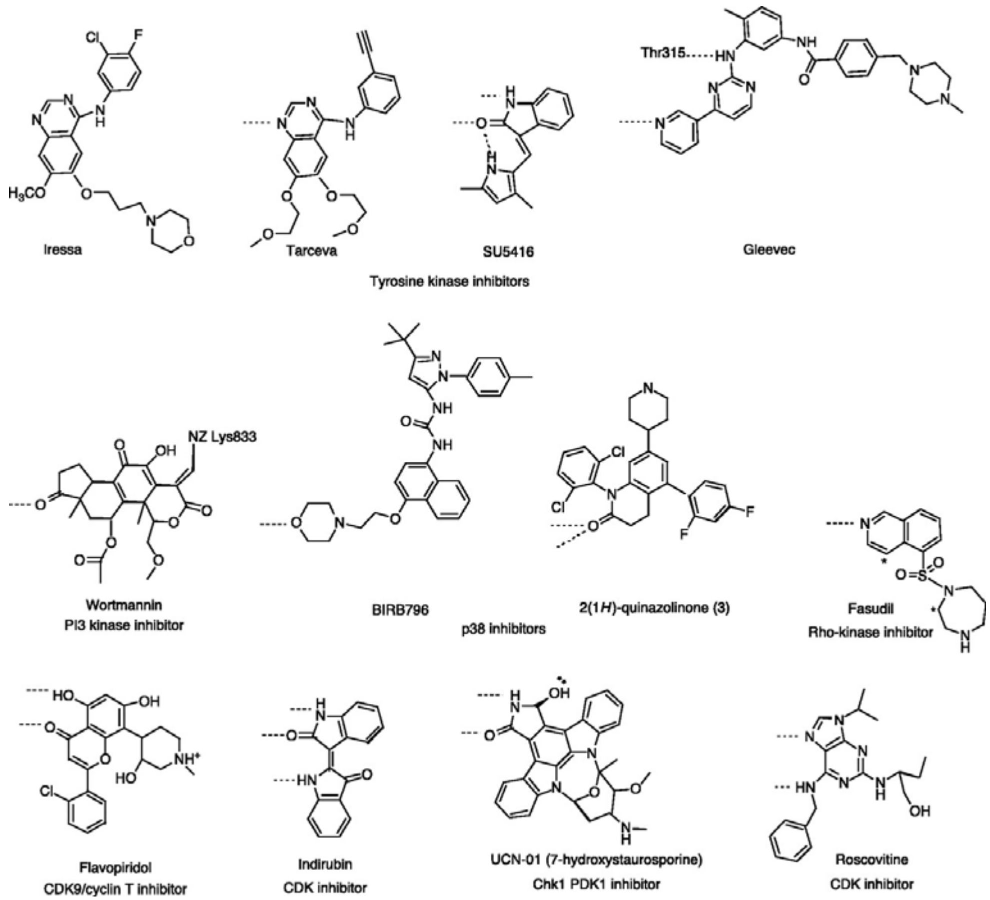
Septicemia



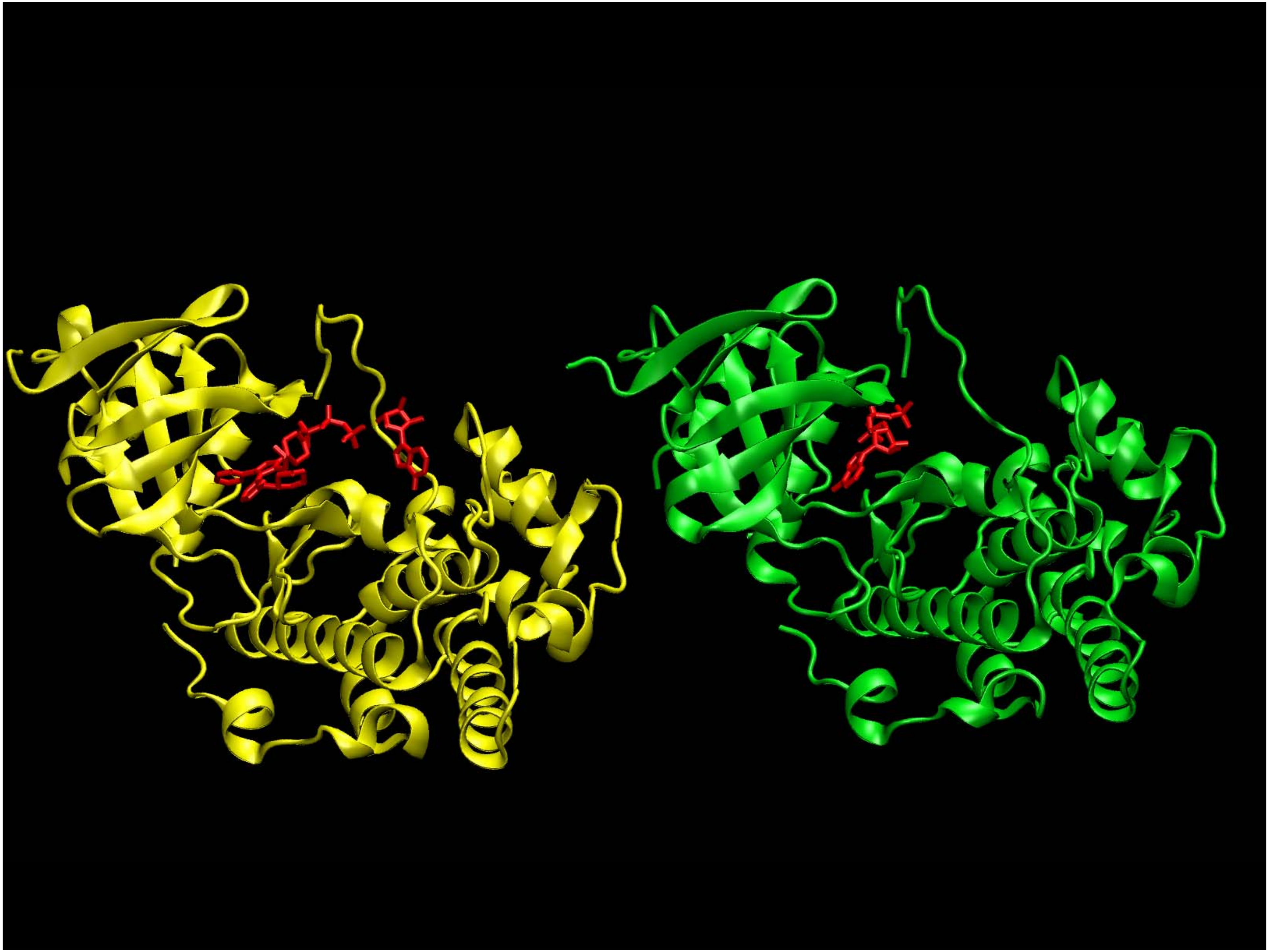
MAP Kinase Regulation of Spore/Macrophage Interaction



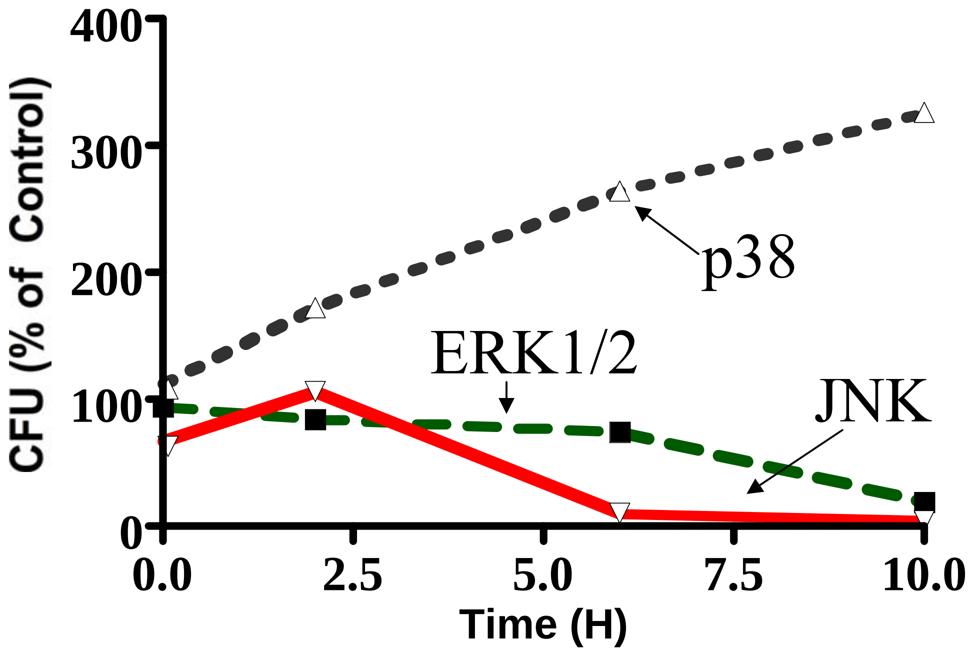
Examples of Protein Kinase Inhibitors



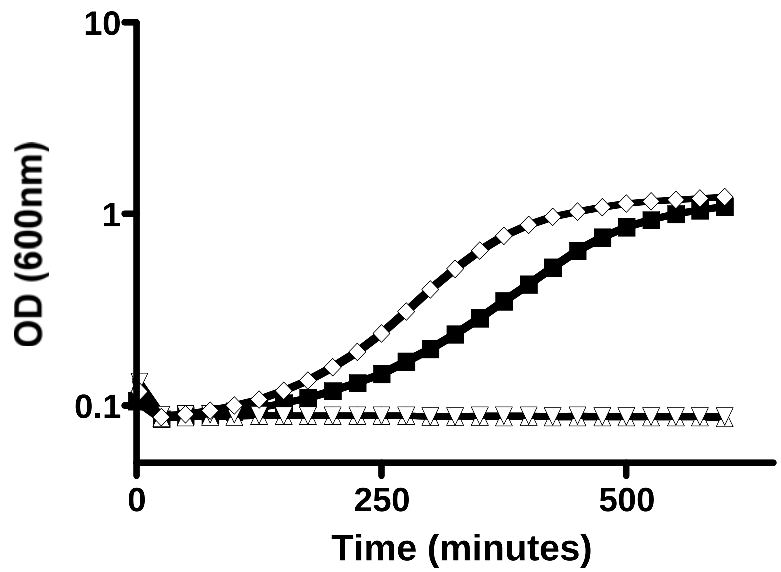
Noble et al. (2004) Protein Kinase Inhibitors: Insights into Drug Design from Structure. *Science,* 303, 1800-1804



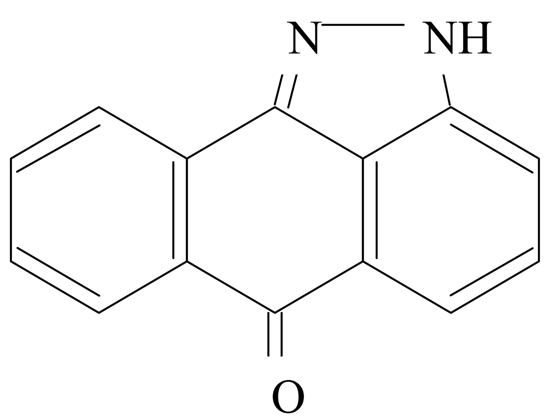
Impact of MAPK Inhibitors on Survival of *B. anthracis* within Macrophages



Impact of c-Jun N-Terminal Kinase (JNK) Inhibitor on *B. anthracis* Growth



JNK-II Inhibitor



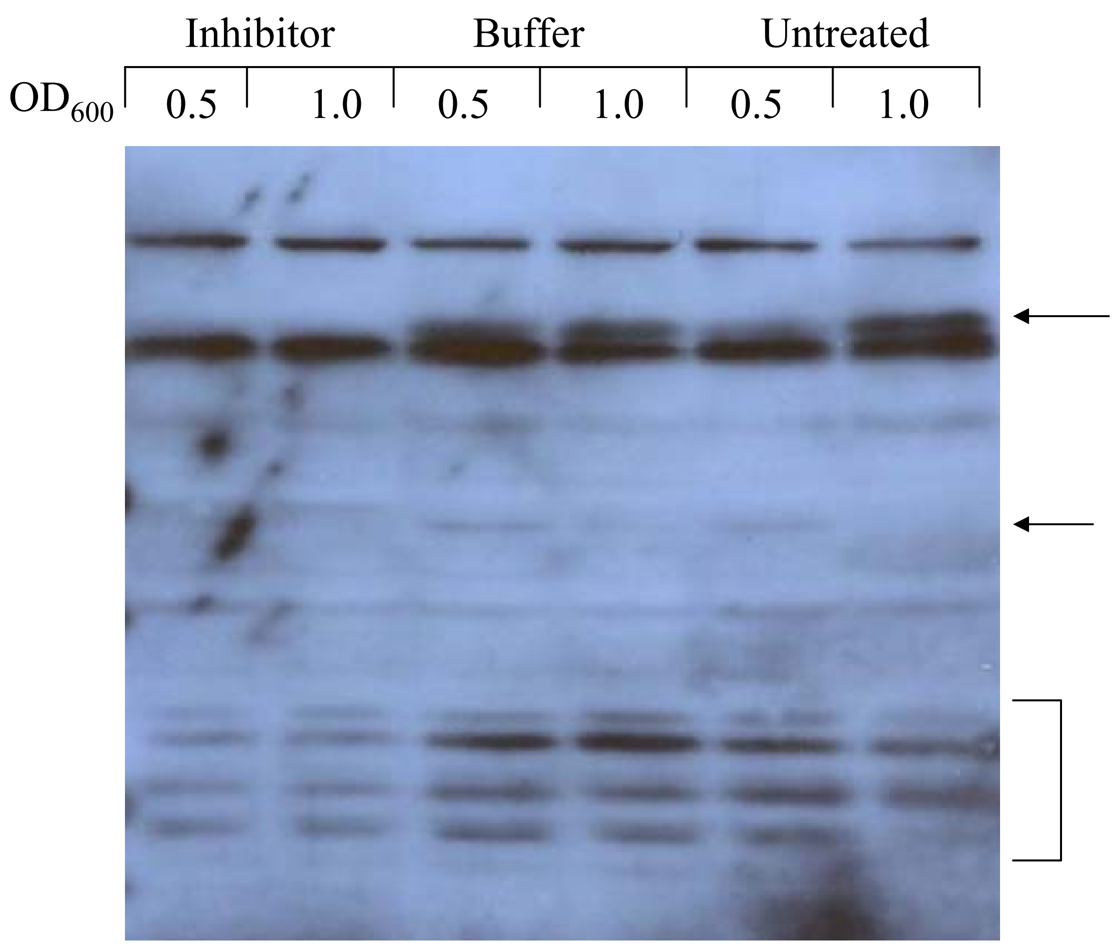
anthra(1,9-cd)pyrazol-6(2H)-one;

1,9-pyrazoloanthrone.

Initial Questions

1. Does the JNK Inhibitor alter protein phosphorylation in *B. anthracis*?
2. Is *B. anthracis* sensitive to other kinase inhibitors?
3. What is the impact of the JNK inhibitor on the growth of other microorganisms?

p-Threonine Profiles



Impact of Kinase Inhibitors on *B. anthracis* Growth

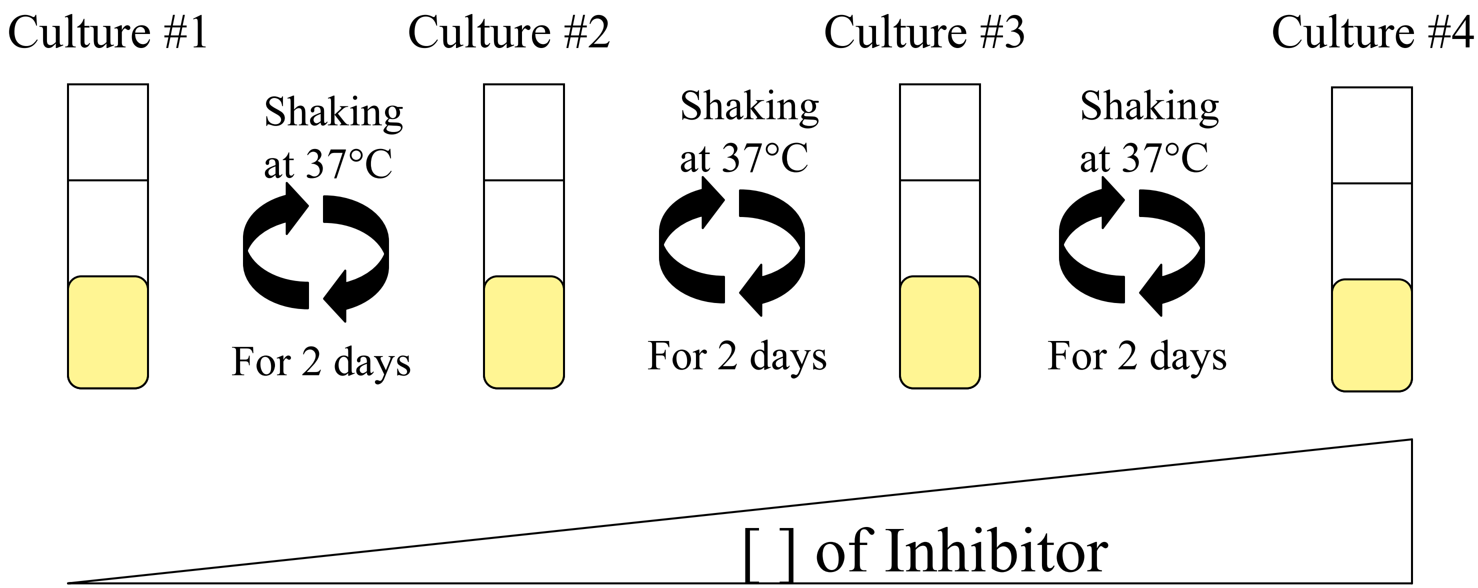
Compound	**Known Eukaryotic Targets**	**Inhibitory Doses**	***B. anthracis* growth**
PD98059	MEK1,2	IC_{50}	+
SB203580	p38	IC_{50} 34 nM	+
JNK-II inhibitor	JNK1,2,3	IC_{50} 40 nM-90 nM	–
Bisindolylmaleimide I	PKCα,β,$\delta\varepsilon$	IC_{50} 8.4 nM-132 nM	+
H-89	PKA, MLCK, CaMKin II, PKC, CKI	K_i 50 nM-40 μM	–
KN-93	CaM Kinase	K_i 370 nM	+
ML-7	MLCK, PKA, PKC	K_i 300 nM-42 μM	+
Protein Kinase G Inh.	PKG, PKA	K_i 85 μM-550 μM	+
Staurosporine	PKA, PKC, PKG, MLCK, CaM Kinase	IC_{50} 0.7 nM-20 nM	+

Organism	MIC/MBC
Escherichia coli 25922	N/N
Salmonella typhimurium	N/N
Enterococcus faecalis	N/N
Listeria monocytogenes	N/N
Bacillus subtilis	320 μM/320 μM
Pseudomonas aeruginosa	N/N
Bacillus cereus	160 μM/160 μM
Staphylococcus epidermidis	N/N
Staphylococcus aureus	N/N
Candida albicans	1280 μM/1280 μM
Bacillus anthracis Sterne 7702	160 μM/160 μM
Streptococcus gordonii	N/N
Streptococcus pyogenes	N/N
Streptococcus pneumoniae	N/N

- Members of the *Bacillus* genus are sensitive to the JNK inhibitor
- Other kinase inhibitors did not have similar effects

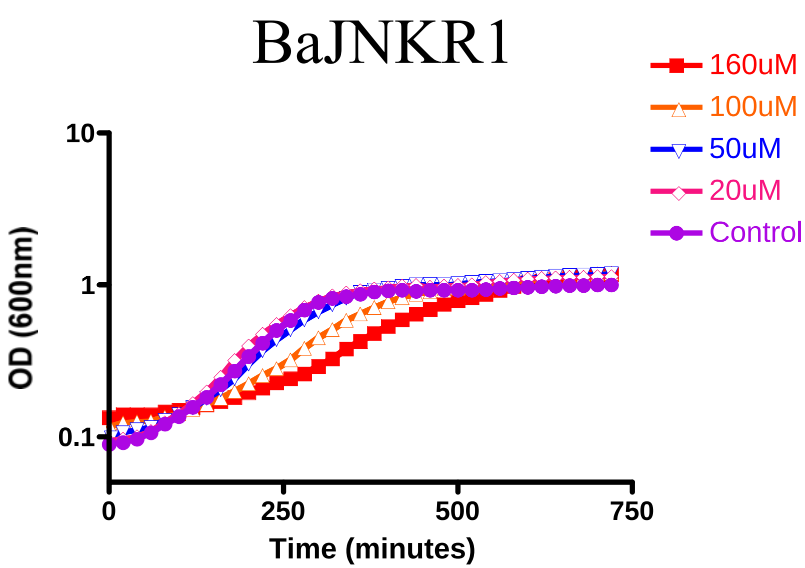
What are the targets of the JNK-inhibitor?

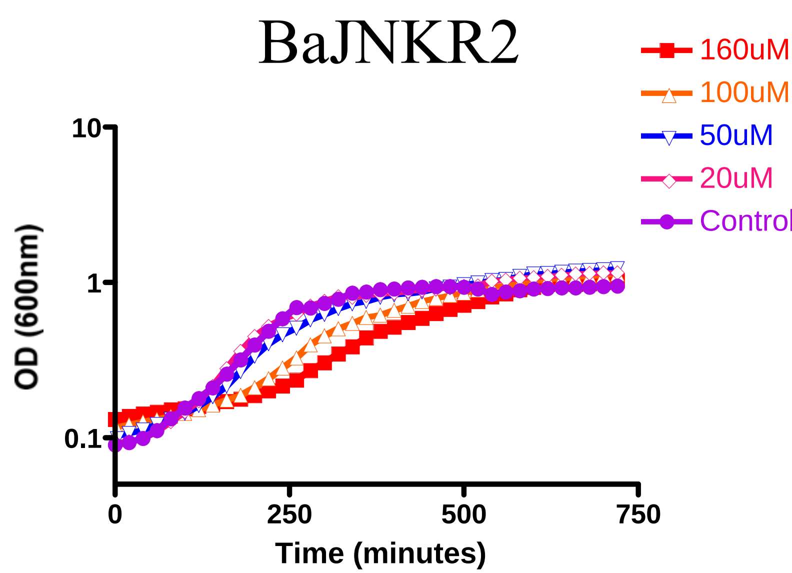
Resistance to JNK Inhibitor



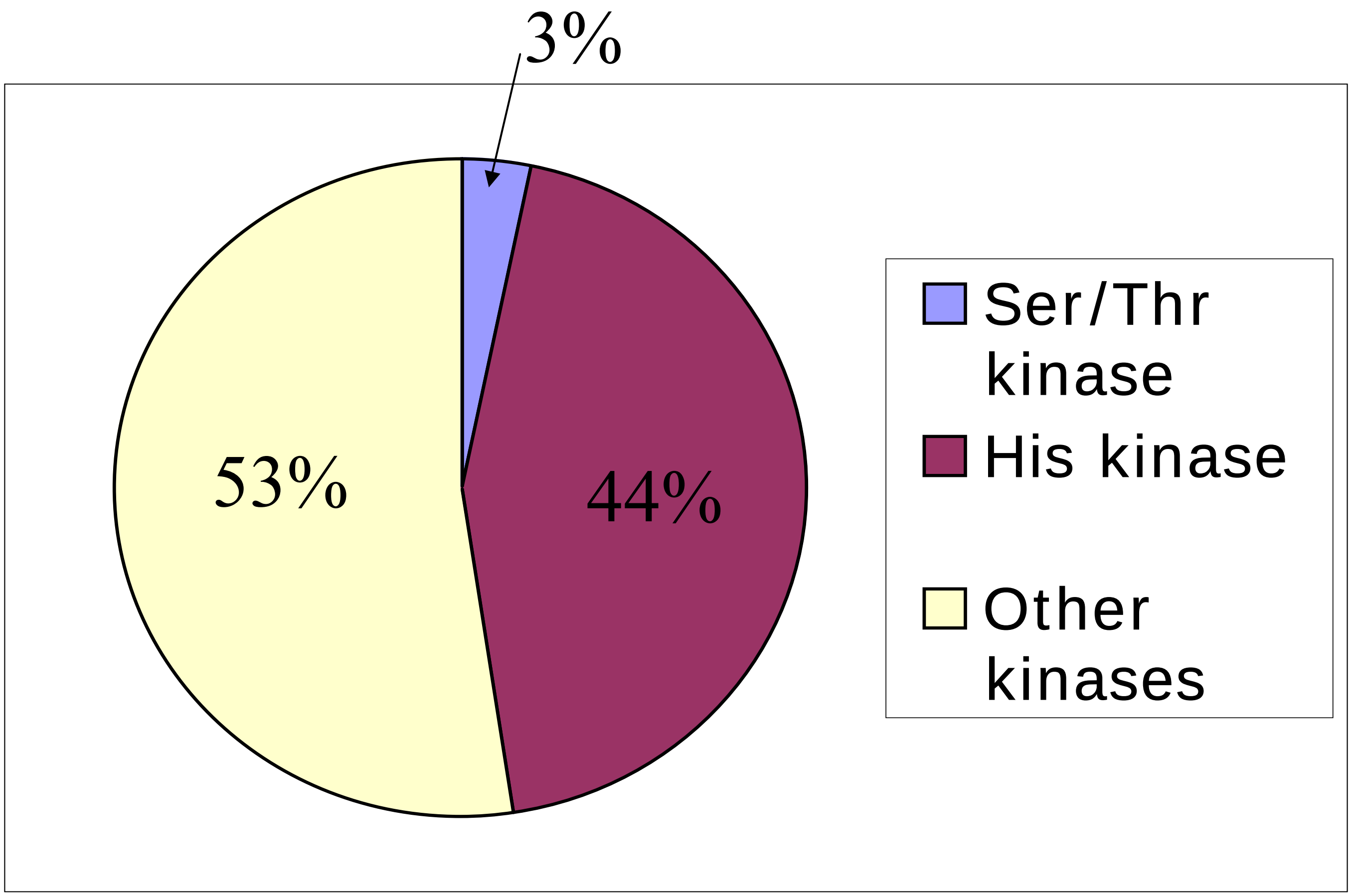
Multiple and/or redundant targets?

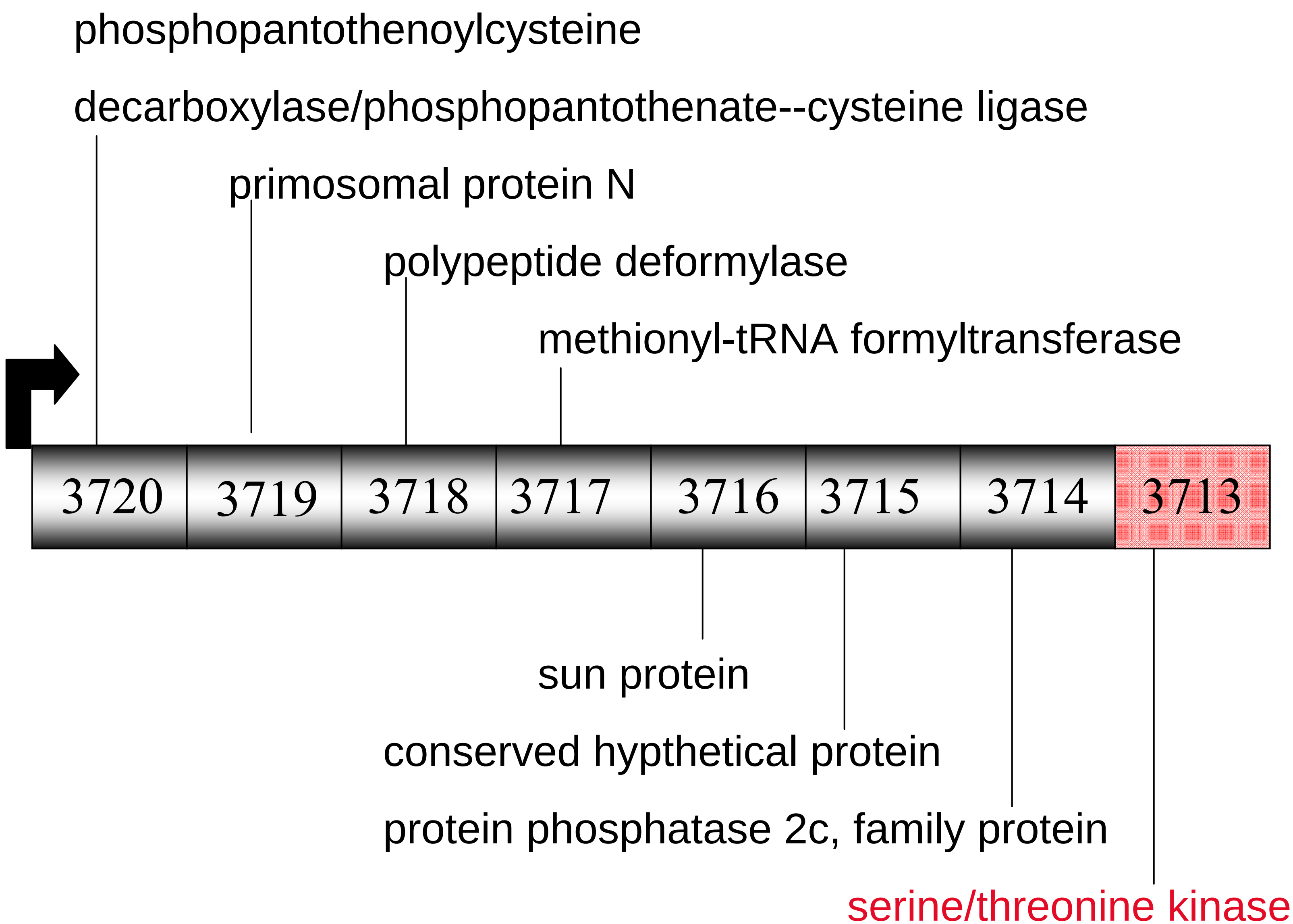
B. anthracis mutants resistant to JNK inhibitor II



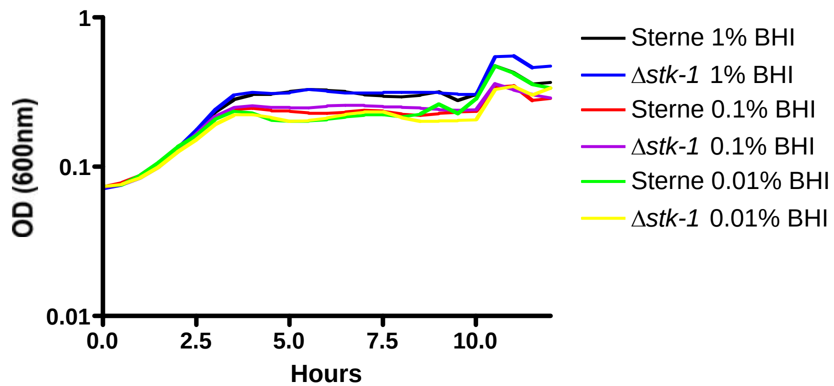


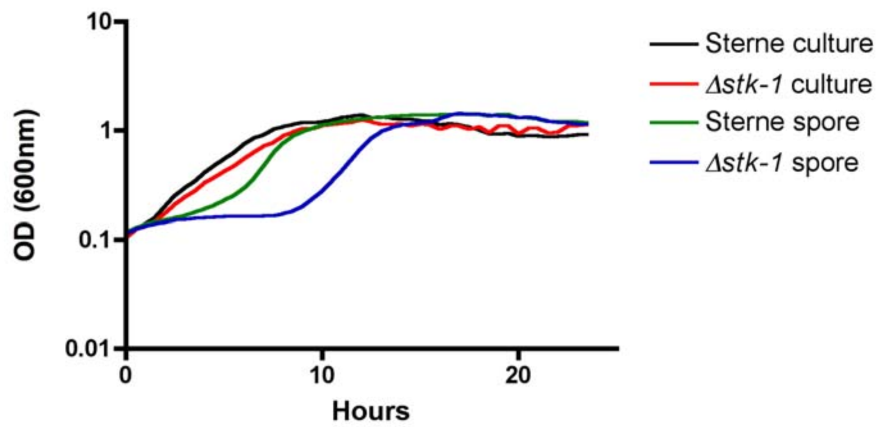
The Kinome of *B. anthracis*



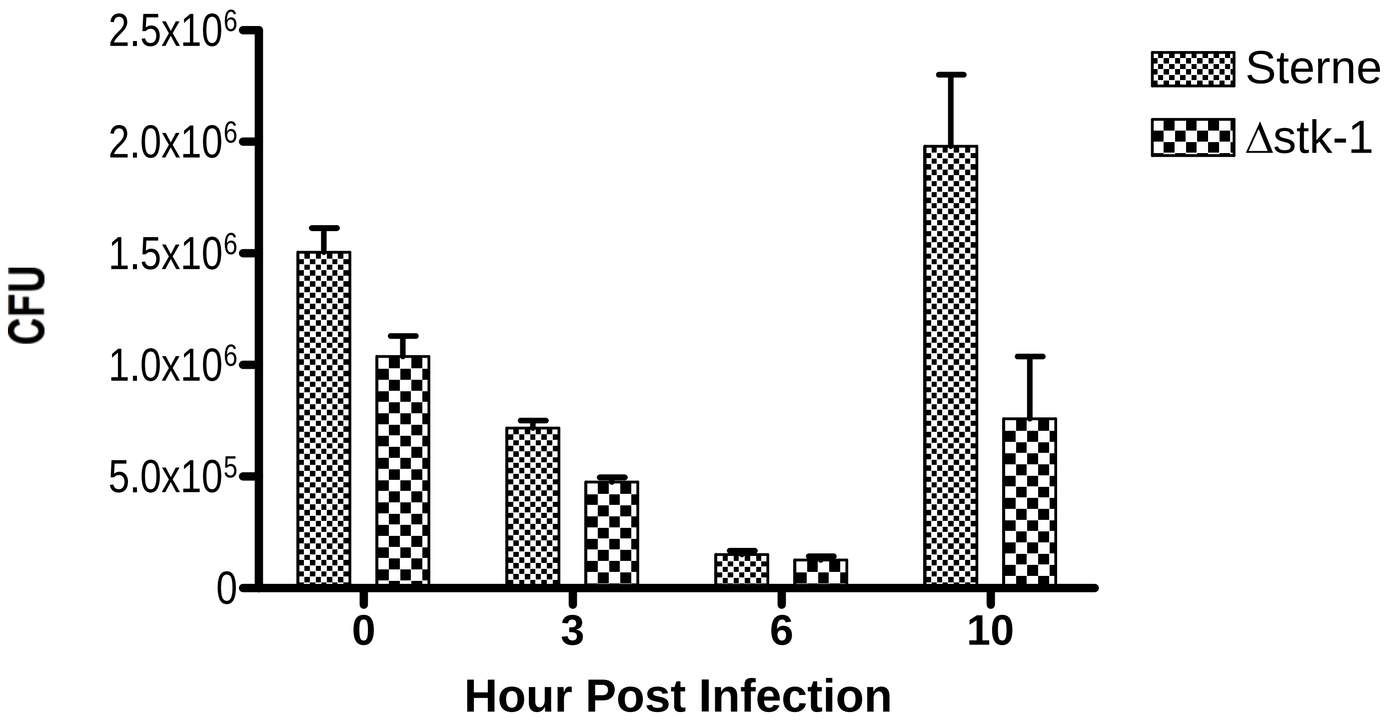

phosphopantothenoylcysteine
decarboxylase/phosphopantothenate--cysteine ligase
primosomal protein N
polypeptide deformylase
methionyl-tRNA formyltransferase
3720
3719
3718
3717
3716
3715
3714
3713
sun protein
conserved hypthetical protein
protein phosphatase 2c, family protein
serine/threonine kinase

Analysis of *B. anthracis* Δ*stk-1*





Macrophage Infection



Significant difference in total cfu at 10 hours post infection

Summary

- *Bacillus anthracis* is sensitive to an inhibitor of c-Jun N-terminal kinase (JNK)
- JNK inhibitor is bactericidal to *B. cereus* and *B. subtilis*, but not against a range of other organisms
- Frequency of resistance is low, and requires multiple passages using shallow increases of inhibitor to obtain resistant isolates
- Candidate targets are under investigation using genetic and biochemical approaches
  - Stk-1 (a kinase homologous to JNK) is necessary for growth under nutrient limiting conditions, and intracellular growth in macrophages

Acknowledgements

- Katie Bryant
- Salika Shakir
- Kevin DeGiusti